**Remarks**

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Initially, applicants would like to thank Examiner Horlick for the courtesy extended to applicant's undersigned representative during the interview on September 8, 2003. The substance of the interview is reflected in the remarks below.

The rejection of claim 15 under 35 U.S.C. § 112 (second paragraph) is overcome by the above amendments and should therefore be withdrawn.

The rejection of claims 1-3 and 16-23 under 35 U.S.C. § 112 (second paragraph) for indefiniteness is respectfully traversed. The U.S. Patent and Trademark Office ("PTO") has maintained its position that the "determining" step as recited in claim 1 is indefinite and that the limitations of claim 4 should be recited in independent claim 1. Applicants respectfully disagree.

The present invention involves the preparation of mutant resistance genes via successive rounds of mutagenesis and selection for enhancement of the resistance phenotype during each of the successive rounds. Typically this is carried out until clinical resistance is achieved (see Example 2 and Table 1, defining clinical resistance minimum inhibiting concentration; and Example 6, description of pAmpC21B as achieving clinical resistance to six drugs). Once the desired level of resistance is achieved, the mutant allele is sequenced to identify the nucleotide and amino acid mutations relative to wild type. Next, the evolutionary potential of the mutant allele is determined (i.e., whether the mutant allele can arise in nature – in response to drug use – by a series of discrete mutations that confer an enhancement of the resistance phenotype).

One approach for making this determination is described in the application and recited in claim 4.

A second approach (identified by the applicants) relies at least in part on the principle of clonal displacement. Because an enhancement of the resistance phenotype is selected during each of the successive rounds of mutagenesis and selection, clonal displacement predicts that the best clone (or several equally competitive clones) will come to dominate the pool of clones. Thus, one of the dominant clones will be selected for mutagenesis during any successive rounds. To the extent there exists only a single mutation between a dominant clone and its ancestor (i.e., the dominant clone of a predecessor generation), it is possible to identify individual evolutionary steps that can occur to attain the

final mutant allele (i.e., from the dominant clone of a final generation). Only when two or more discrete mutations occur in a single round of mutagenesis is it necessary to assess which of the two or more possible evolutionary pathways can arise in nature to attain the final mutant allele. Thus, according to this approach, sequencing of the dominant clones selected after each round will allow for determining whether the mutant resistance gene is likely to evolve in nature and the extent to which the steps recited in dependent claim 4 need to be performed, if at all.

This second approach is described in detail in Hall and Barlow, "Experimental Prediction of the Evolution of Cefepime Resistance From the CMY-2 AmpC β-Lactamase," *Genetics* 164:23-29 (2003) (copy attached hereto as Exhibit A) at pages 27-28, where the evolutionary potential of a mutant CMY-2 allele possessing five discrete amino acid substitutions was demonstrated to be likely to evolve in nature.

As demonstrated above, a number of approaches can be used to determine whether or not the mutant resistance gene is likely to evolve through two or more independent mutation events, where each independent mutation event confers an enhancement of the resistance phenotype. For this reason, one of ordinary skill in the art would understand that the "determining" step recited in claim 1 is not indefinite. Therefore, the rejection of claims 1-3 and 16-23 for indefiniteness should be withdrawn.

The rejection of claims 1-10, 12, 13, and 15-43 under 35 U.S.C. § 103(a) for obviousness over U.S. Patent No. 5,766,842 to Melnick et al., U.S. Patent No. 6,063,562 to Melnick et al., or PCT Application Publ. No. WO 96/08580 to Melnick et al. (collectively "Melnick") is respectfully traversed.

Melnick concerns the preparation of all first-generation mutants that may emerge in response to the clinical use of a particular microbial agent. The first-generation mutants can include one or more (preferably not more than three) amino acid substitutions. Melnick sequences all of the first-generation mutants to identify all the substitutions present in the first-generation mutants. As shown in Figure 1 of Melnick, Melnick thereafter deconstructs the population of first-generation mutants by preparing and testing all possible single amino acid mutants to determine which can be responsible for the enhanced resistance phenotype, identified as *1st new mutants*. (To the extent that any of those single amino acids were present alone in any of the first-generation mutants, that in and of itself confirms the ability of that particular mutation to enhance the resistance phenotype.) For 1st new mutants that *do not* achieve enhanced resistance, Melnick forms *2nd new mutants* that contain two amino acid substitutions in combination, neither of which alone had an effect on phenotype.

(This aspect of Melnick is distinct of forming multiple mutants where each independent mutation event confers enhancement of the resistance phenotype.)

Because Melnick is concerned with preparing mutant resistance genes so that drugs and drug combinations can be identified that will prevent development of all the first-generation mutants, Melnick is not at all concerned with the development of mutant resistance genes that develop through multiple rounds of mutagenesis and selection until clinical resistance is achieved. Moreover, Melnick makes no attempt to assess whether mutant alleles possessing two or more mutations can evolve in nature, where *each independent mutation event confers an enhancement of the resistance phenotype.* Melnick assesses whether single mutations can give rise to the resistance phenotype and, if not, whether two or more silent mutations can enhance the resistance phenotype when combined (see *2nd new mutants* in Figure 1 of Melnick).

The PTO has asserted that Melnick nonetheless suggests the "determining" step recited in claim 1 given that Melnick discloses "creating a new first and/or second set of mutants" (see office action at page 7) and teaches "the detection of 'combination' resistance-conferring mutations involving two or more amino acid substitutions, and repetitive screening procedures to test all possible combinations of substitutions for resistance conferring properties" (id.). For the reasons noted above, the PTO's position is misplaced given that Melnick fails to teach or suggest performing multiple rounds of mutagenesis and selection let alone determining whether resulting mutant alleles that possess two or more mutations can evolve in nature, where *each independent mutation event confers an enhancement of the resistance phenotype.* Melnick actually teaches away from the formation of mutant alleles possessing two or more mutations that are prepared via multiple rounds of mutagenesis and selection until clinical resistance is achieved. Melnick further teaches away from the above-noted "determining" step as recited given that Melnick never attempts to form a mutant allele that possesses two or more substitutions each of which confers an enhancement of the resistance phenotype. This is evident from the final set of resistance-conferring mutations identified in Figure 1 of Melnick, only one of which includes two mutations but neither of which independently confers enhancement of the resistance phenotype.

Because Melnick fails to teach or suggest the above-noted limitations, Melnick cannot have rendered the invention of claims 1-10, 12, 13, and 15-34 obvious under 35 U.S.C. § 103(a).

With respect to claims 35-43, applicants submit that Melnick cannot have rendered the claimed invention obvious. As noted in the response submitted June 19, 2002, Melnick, while concerned with assessing drug longevity, teaches making the assessment by

determining the number of "distinct, first-generation, drug-resistant, biologically active mutants that emerge in response to the use of the drug" (see, e.g., Melnick '842 patent at col. 8, line 56 to col. 9, line 2). Thus, Melnick suggests that drug longevity is inversely related to the sheer number of first-generation mutants. In sharp contrast, the invention of claims 35-43 concerns assessing the longevity of a drug instead by "determining the minimum number of mutations required to overcome the activity of the candidate anti-pathogenic drug, wherein the greater the minimum number of mutations, the greater the potential longevity of the candidate anti-pathogenic drug." Nowhere does Melnick teach or suggest making such a measurement. Thus, Melnick, while purportedly providing the same assessment, does so in an entirely different manner that would not have suggested the steps recited for making the assessment in accordance with the present invention.

Because Melnick fails to teach or suggest the above-noted limitations, Melnick cannot have rendered the invention of claims 35-43 obvious under 35 U.S.C. § 103(a).

For all these reasons, the rejection of claims 1-10, 12, 13, and 15-43 is improper and should be withdrawn.

In view of all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

Date: October 8, 2003

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